

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
22 March 2001 (22.03.2001)

PCT

(10) International Publication Number  
WO 01/19792 A1

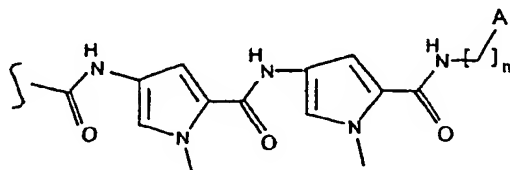
- (51) International Patent Classification<sup>7</sup>: C07D 207/34, 403/14, 409/14, 405/14, C12N 15/09 (74) Agents: GORTHEY, LeeAnn et al.; Iota Pi Law Group, P.O. Box 60850, Palo Alto, CA 94306-0850 (US).
- (21) International Application Number: PCT/US00/25325 (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.
- (22) International Filing Date: 15 September 2000 (15.09.2000)
- (25) Filing Language: English
- (26) Publication Language: English (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- (30) Priority Data: 60/154,415 17 September 1999 (17.09.1999) US
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Published:

- With international search report.
- Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: COVALENTLY LINKED DIMERIC DNA BINDING MOLECULES



(I)

(57) Abstract: DNA binding compounds which are dimers of netropsin or netropsin analogs, linked by a polycyclic linker group, are disclosed. The binding compounds have the structure B-L-B', where B and B' independently have the structure (I) in which A is amidinyl (-C(NH<sub>2</sub>)=NH) or -CH<sub>2</sub>NRR', where R and R' are

independently hydrogen or lower alkyl, or together form a 5- to 7-member heterocyclic ring whose ring atoms are selected from the group consisting of carbon, nitrogen, oxygen and sulfur, and include at least 3 carbon atoms, and n is 1-6. L is a fused polycyclic ring system containing at least one aromatic ring, and B and B' are linked to different rings of said ring system, such that the shortest path between B and B' comprises a sequence of at least three bonds within the ring system, when the groups are attached to adjacent (fused) rings, or at least five bonds within the ring system, when the groups are attached to non-adjacent rings.

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## Covalently Linked Dimeric DNA Binding Molecules

### Field of the Invention

The present invention relates to DNA binding compounds which are dimers of netropsin or  
5 netropsin analogs, linked by a polycyclic linker group.

### Background of the Invention

The binding of the antiviral antibiotics netropsin and distamycin to AT-rich sequences in the  
minor groove of double stranded DNA is a well studied phenomenon. Because such binding can  
10 be used to regulate DNA expression, e.g. by blocking and/or displacement of regulatory  
proteins, or by inhibiting the activity of enzymes acting on DNA, such as reverse transcriptase  
or topoisomerase, optimization of this binding has been the subject of numerous recent studies.

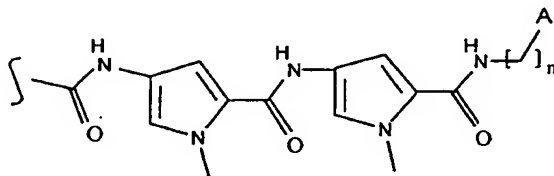
As described in a recent review by Bailly and Chaires (*Bioconj. Chem.* 9(5):513-38, 1998),  
the pyrrolicarboxamide unit in netropsin and distamycin is actually about 20% longer than  
15 required to perfectly match the corresponding base pair sequence in the minor groove.  
Accordingly, in oligomeric analogs having multiple binding moieties, successive binding  
moieties can become out of phase with the base pairs of the minor groove. Several studies have  
therefore been directed to dimers of netropsin or distamycin containing different linkers, in  
order to improve binding to longer target sequences. See, for example, M. Filipowsky *et al.*,  
20 *Biochemistry* 35:15397-410 (1996), Z. Wang *et al.*, *Biochem. Pharmacol.* 53:309-16 (1997),  
and N. Neamati *et al.*, *Mol. Pharmacol.* 54:280-90 (1998).

Linkers employed in these studies included p-phenylene, *trans*-vinyl, cyclopropyl, 3,5-  
pyridyl, and six- and eight-carbon aliphatic chains. Several of these linkers restrict rotation  
around the linking group, thus reducing the extent of purely monodentate binding (i.e. by only  
25 one netropsin moiety) which can occur with flexible linkers. However, Kissinger *et al.* (*Chem.*  
*Res. Toxicol.* 3(2):162-8, 1990) reported that aryl-linked groups had reduced DNA binding  
affinity compared to alkyl and alkylene linkers, and Neamati *et al.* (cited above) reported that  
the *trans*-vinyl linked compound was many times more potent (in inhibiting HIV-1 integrase)  
than the "more rigid" cyclobutanyl and norbornyl linkers. It was suggested in Wang and in  
30 Bailly that, for certain applications, the more rigid linkers (cyclopropyl and p-phenylene, in  
these studies) may not allow for optimal simultaneous (bidentate) binding of the two netropsin  
moieties flanking the linker. Therefore, it would be desirable to provide linkers which provide  
suitable geometries for bidentate binding.

### Summary of the Invention

The present invention includes, in one aspect, a DNA binding compound having the

structure B-L-B', where B and B' are independently represented by the structure:



in which

A is amidinyl ( $-C(NH_2)=NH$ ) or  $-CH_2NRR'$ , where R and R' are independently hydrogen or lower alkyl, or together form a 5- to 7-member heterocyclic ring whose ring atoms are selected from the group consisting of carbon, nitrogen, oxygen and sulfur, and include at least 3 carbon atoms;

n is 1-6;

L is a fused polycyclic ring system containing two or more fused 5- to 7-membered rings, and containing at least one aromatic ring;

and B and B' are linked to different rings of said ring system, such that the shortest path connecting said groups B and B' comprises at least three contiguous bonds within the ring system when the two groups are on adjacent fused rings, and comprises at least five contiguous bonds within the ring system when the two groups are on non-adjacent (non-fused) rings. These contiguous bonds are preferably part of a conjugated pi system.

In preferred embodiments, the ring system is bicyclic or tricyclic, the groups A are amidinyl or (dimethylamino)methyl, and  $n=2$ . In further specific embodiments, the linker L is 2,5-indolyl or 9,10-dihydro-2,7-phenanthrenyl. Other preferred linkers include 2,7-phenanthrenyl, 3,7-dibenzofuranyl and 3,7-dibenzothiophenyl. For ease of preparation, B and B' are typically identical groups.

Also provided is a method of regulating DNA expression, in which a compound of structure B-L-B', as defined above, is contacted with DNA, preferably double stranded DNA, such that the compound binds to the DNA in a manner effective to promote or inhibit expression of the DNA. The compound may be effective to block and/or displace a regulatory protein or to inhibit the activity of an enzyme acting on DNA, e.g. reverse transcriptase or topoisomerase.

These and other objects and features of the invention will become more fully apparent when the following detailed description of the invention is read in conjunction with the accompanying drawings.

**Brief Description of the Drawings**

Figures 1A-1H show UV absorption spectra of selected compounds of the invention;

Figure 2 is a graphical analysis of a footprinting gel for invention compound **21**, run on a DNA sequence (SEQ ID NO: 1) derived from human IL-1 $\alpha$ , showing observed regions of partial or strong footprinting, as indicated, and the concentrations of binding compound and control compound. In this Figure and Figures 3A through 6B, concentrations of control compound (Distamycin, represented by **D**) and invention compound (in each case represented by **C**) are given along the left edge of the Figures in  $\mu\text{M}$ ; e.g., in Fig. 2, "C 0.1" indicates 0.1  $\mu\text{M}$  of invention compound **21**.

Figures 3A-B are similar graphical analyses of a footprinting gel for invention compound **21**, using a DNA sequence (SEQ ID NO: 2) derived from human IL-1 $\beta$ ;

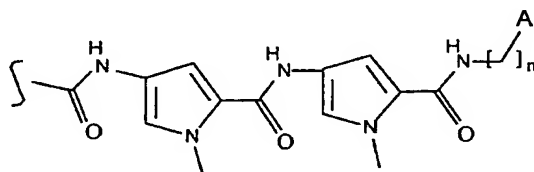
Figure 4 is a graphical analysis of a footprinting gel for invention compound **21**, using a DNA sequence (SEQ ID NO: 3) derived from human IL-2;

Figure 5 is a graphical analysis of a footprinting gel for invention compound **25**, using a DNA sequence (SEQ ID NO: 1) derived from human IL-1 $\alpha$ ; and

Figures 6A-B are graphical analyses of a footprinting gel for invention compound **25**, using a DNA sequence (SEQ ID NO: 2) derived from human IL-1 $\beta$ .

**Detailed Description of the Invention****I. Netropsin Dimers****A. Structures**

The DNA binding netropsin dimers, or analogs, of the invention have a structure represented by B-L-B', where B and B' each independently represent a netropsin-based structure as shown:



Typically, B and B' are the same in a given compound.

The group A, linked to the dipeptidyl group by an alkyl chain having 1-6 carbons ( $n = 1-6$ ), and preferably having 2 carbons, is one which exists appreciably in protonated form at physiological pH, and, in one embodiment, is amidinyl ( $-\text{C}(\text{NH}_2)=\text{NH}$ ). "A" may also be an aliphatic or alicyclic amine, represented by  $-\text{CH}_2\text{NRR}'$ , where R and R' are independently hydrogen or lower alkyl or together form a 5- to 7-member heterocyclic ring whose ring atoms

are selected from the group consisting of carbon, nitrogen, oxygen and sulfur, and include at least 3 carbon atoms. In this case, a preferred embodiment of A is (dimethylamino)methyl ( $-\text{CH}_2\text{N}(\text{CH}_3)_2$ ). Examples of heterocycle-containing embodiments of A include (1-morpholinyl)methyl, (1-piperazinyl)methyl, and (4-methyl-1-piperazinyl)methyl.

5 The linker L is a fused polycyclic ring system containing at least one aromatic ring. In selected embodiments, L is a bicyclic or tricyclic ring system. Examples of such linkers include 2,5-indolyl, 2,6-indolyl, 2,7-phenanthrenyl, 9,10-dihydro-2,7-phenanthrenyl, 3,7-dibenzofuranyl, 3,7-dibenzothiophenyl, 3,7-fluorenyl, 3,7-fluorenonyl, 2,5-benzimidazolyl, and 2,6-benzimidazolyl. Also contemplated is the cyclic phosphate of 2,2'-biphenyldiol (6-hydroxy-10 dibenzo[d,f][1,3,2] dioxaphosphepin-6-oxide). The ring system typically contains ring atoms selected from the group consisting of carbon, nitrogen, oxygen, and sulfur. In selected embodiments, all of the ring atoms are carbon (i.e. a polycyclic hydrocarbon), or they are selected from carbon and nitrogen (i.e. a carbon-nitrogen heterocycle).

In the compounds disclosed herein, the linking group is substantially rigid, although some 15 conformational flexing may occur (e.g. in 9,10-dihydrophenanthrene), and it is preferably planar or nearly planar. Further, each binding group B and B' (specifically, the leftmost carbonyl group in the structure shown above, in each group B and B') is linked to a different ring of the polycyclic ring system. When the two groups are on adjacent (fused) rings, the shortest path connecting the two groups comprises at least three contiguous bonds within the ring system. 20 When they are on non-adjacent (non-fused) rings (e.g. the 1 and 3 rings in a tricyclic ring system), the shortest path connecting the two groups comprises at least five contiguous bonds within the ring system. These contiguous bonds are preferably part of a conjugated pi system.

The connecting path lengths so defined provide that the bonds connecting the linker L to the two netropsin-based moieties B and B' are disposed in a linear or approximately linear 25 arrangement; that is, the angle between these bonds (in a plane roughly defined by the linker moiety) is preferably greater than  $90^\circ$ , and more preferably between about  $135^\circ$  and  $180^\circ$ . Accordingly, linkers such as 1,8-naphthyl or 1,8-anthracyl (where the linking bonds would be essentially parallel) or 3,4-indolyl (where the linking bonds would be nearly parallel) would not be included.

30 Because the groups B and B' are linked to different rings in the polycyclic ring system, as described above, the linkage is longer than those shown in the prior art based on e.g. phenyl, vinyl, pyridyl, or cycloalkyl groups. This longer spacing is expected to accommodate binding to target sequences for which these shorter linkers are less optimal. As shown below, the dimeric compounds bind to DNA with an affinity that is often much greater than that shown by the 35 monomeric counterparts, and in some cases with differing sequence selectivity.

Also contemplated are dimers of distamycin or lexitropsin analogs employing the polycyclic linkers described herein; i.e. where the groups represented by B and B' have an additional pyrrolicarboxamide subunit (distamycin and analogs thereof) or wherein one or more of the pyrrole rings is replaced with imidazole (lexitropsin and analogs thereof).

5       B. Synthesis

The compounds can be prepared by condensation of two equivalents of a dipyrrole peptide, as described below, with a dicarboxylic acid of the form HOOC-L-COOH, where L is as defined above, or an activated derivative thereof such as a diacid chloride or diimidazolidine. The dipyrrole peptide has an amino group at the 3-position of the first pyrrolamide moiety. Linked  
10 to the amide nitrogen of the terminal pyrrolamide moiety is a group of the form  $-(CH_2)_nY$ , where Y is a cyano group, an alkylamino methyl group, or  $-CH_2X$ , with X representing an efficient leaving group such as halogen. (In this sense, "alkylamino" can include nitrogen-containing heterocycles such as piperazine or morpholine.) The value of n can range from 1 to 6, and is typically 2. Preparation of such dipeptides is described, for example, in Lown &  
15 Krowicki (*J. Org. Chem.* 50:3774-79, 1985) or in Nishiwaki *et al.* (*Heterocycles* 27(8):1945-52, 1988).

For preparing specific embodiments of the present compounds, the following dicarboxylic acids, or derivatives thereof, can be used: indole-2,5-dicarboxylic acid, indole-2,6-dicarboxylic acid, 2,7-phenanthrenedicarboxylic acid, 9,10-dihydrophenanthrene-2,7-dicarboxylic acid,  
20 fluorene-2,7-dicarboxylic acid, fluorenone-2,7-dicarboxylic acid, 3,7-dibenzofurandicarboxylic acid, and 3,7-dibenzothiophenedicarboxylic acid. Such compounds, as well as other diacids suitable for preparing compounds within the scope of the present claims, are frequently commercially available or can be prepared by one of ordinary skill in the art according to known synthetic methods. For example, differently substituted 2-carboxyindoles can be prepared  
25 according to the method of D. Jones *et al.*, U.S. Patent No. 3,838,167 (1974); 2,5- and 2,6-substituted indoles can be synthesized by modified Fischer indole syntheses and further elaboration of the nucleus, as reported in A. I. Khalaf *et al.*, *J. Chem. Res.* 751-54 (2000). Dibenzofuran-3,7-dicarboxylic acid can be prepared by air oxidation of the corresponding dimethyl dibenzofuran (T. Kaneda *et al.*, JP Kokai No. 53-12988 (1978); methyl-substituted  
30 dibenzofurans can in turn be efficiently prepared by dilithiation-oxidative coupling of methyl-substituted diphenyl ethers (F. Radner *et al.*, *J. Chem. Res. Synop.* 8:362-62 (1996). M. Dotrong *et al.* (*J. Polym. Sci., Part A: Polym. Chem.* 28(12), 3241-50, 1990) describe the preparation of fluorene-2,7-dicarboxylic acid from commercially available 2,7-dibromofluorene, via the dinitrile. Similarly, 9,10-dihydrophenanthrene-2,7-dicarboxylic acid can be prepared by  
35 prepared by Friedel-Crafts acetylation of 9,10-dihydrophenanthrene, followed by iodine

oxidation and aqueous base hydrolysis. An alternate route employs bromination of 9,10-dihydrophenanthrene, followed by Pd-catalyzed carbonylation (A.D. Abell *et al.*, *J. Chem. Soc. Perk. Trans. I* 11:1663-7, 1997). In a preparation of fluorenone-2,7-dicarboxylic acid reported by Hodson *et al.* (U.S. Patent No. 3,987,088), fluorene is acetylated with  $\text{Ac}_2\text{O}/\text{AlCl}_3$  to give  
5 2,7-diacetylfluorene, which is then oxidized to the final product.

The condensation reaction is carried out according to standard methods. In a typical procedure, the aminopyrrole dipeptide component is dissolved in anhydrous DMF, the solution is cooled to a temperature of about  $-30^\circ\text{C}$  to  $+10^\circ\text{C}$ , and the diacid chloride, in the presence of a base such as pyridine, triethylamine or diisopropylethylamine, or the diimidazolidine is added  
10 dropwise in anhydrous THF. The reaction is stirred, with warming to room temperature if necessary, until completion; the solvent is removed and the residue purified by conventional techniques.

For preparation of amidino-terminated dimers, a cyano-terminated aminopyrrole dipeptide is employed. After condensation with an activated N-methyl-4-nitropyrrole-2-carboxylic acid  
15 monomer to give the nitrodimer, subsequent reaction with  $\text{HCl}/\text{ethanol}$ , followed by  $\text{NH}_3/\text{ethanol}$ , as described in U.S. Patent No. 5,616,606, gives the amidinium chloride.

Hydrogenation over palladium on charcoal then gives the required aminodimer (see Example 1).

For preparation of various aliphatic or alicyclic amine-terminated dimers, an activated form of N-methyl-4-nitropyrrole-2-carboxylic acid is reacted with the appropriate substituted aliphatic  
20 amine (the substituent being, for example, morpholino, N-methylpiperidinyl, pyrrolidinyl, bis(2-hydroxyethyl)amino, dimethyl- or diethylamino), to give the nitro monomer. This compound is hydrogenated over palladium on charcoal and coupled to another activated N-methyl-4-nitropyrrole-2-carboxylic acid monomer to give the nitro dimer, which is the hydrogenated to furnish the amine for coupling to the linking group.

25 A series of invention compounds, as shown in Figs. 1A-H, were prepared and were characterized using NMR and UV spectroscopy. The following UV absorption data (solvent = DI water) was obtained for representative compounds of the invention. Proton NMR data for compound **21** is given in Example 1.

UV Absorption Data

Cmpd No.	$\lambda_{\text{max}}$	$\epsilon$	$\lambda_{\text{max}}$	$\epsilon$	$\lambda_{\text{max}}$	$\epsilon$
21	258	40640	210	42160		
24	194	39259	272	59056		
25	308	50793	330	50869		
26	240	47163	310	46741	334	48235
31	248	37123	330	38095		
32	244	51394	310	42853		
43	202	40997	244	28048	304	38655
46	260	47858	300	45582		
49	220	43042	308	48126		

II. DNA Binding Assays

5 Compounds of the invention exhibit sequence-specific or sequence-preferential binding and high binding affinity to DNA sequences, particularly A/T rich sequences. Binding of invention compounds to several DNA sequences was examined using nuclease footprinting, a technique well known in the art. Briefly, in footprinting, the region of DNA that is bound to a binding compound is protected from nuclease degradation. When samples are digested in the presence and absence of the binding compound, the sequence to which the compound binds appears as a gap, or footprint, in the array of bands obtained on electrophoresis. Preferably, several such experiments are run in parallel, using increasing amounts of the binding compound, in order to evaluate binding affinity.

15 Several compounds in accordance with the invention, as shown in Table 1, were examined for binding affinity to sequences derived from IL-2 $\alpha$  DNA. Experiments were also run using compounds of the prior art, i.e. netropsin dimers linked by *trans*-vinyl, cyclopropyl, or m-phenylene linkers (designated 45, 48, and 50, respectively). Selected compounds were also tested for binding to sequences derived from IL-1 $\alpha$ , IL-1 $\beta$ , and  $\gamma$ -interferon DNA. The dimeric compounds were run side-by-side with a corresponding monomeric binding compound such as netropsin or distamycin.



**Table 1**

No.	Linker L	End Groups B, B' (n=2)
21	2,5-indolyl (2,5-I)	-C(NH <sub>2</sub> )=NH (AM)
24	3,7-fluorenonyl	(dimethylamino)methyl (DMA)
25	9,10-dihydro-2,7-phenanthrenyl (2,7-DHP)	AM
26	2,7-DHP	DMA
31	2,6-indolyl (2,6-I)	DMA
32	3,7-dibenzothiophenyl	DMA
43	6-hydroxy-dibenzo[d,f][1,3,2]dioxaphosphepin-6-oxide	DMA
46	2,7-phenanthrenyl (2,7-PHE)	DMA
49	3,7-dibenzofuranyl	DMA
51	2,7-DHP	(1-morpholinyl)methyl
52	2,7-DHP	(4-methyl-1-piperazinyl)methyl
45	<i>trans</i> -vinyl	DMA
48	1,2-cyclopropyl	DMA
50	<i>m</i> -phenyl	DMA

Graphical analyses of the footprinting gels, showing observed regions of partial or strong footprinting, as indicated in the Figures, are included in Figs. 2-4 for compound 21 (DNA sequences having SEQ ID NOs: 1-3, derived from human IL-1 $\alpha$ , IL-1 $\beta$ , and IL-2, respectively) and in Figs. 5-6 for compound 25 (DNA sequences having SEQ ID NOs: 1-2, derived from IL-1 $\alpha$  and IL-1 $\beta$ , respectively). As noted above, concentrations of control compound Distamycin, represented by D, and invention compound, in each case represented by C, are given along the left edge of the Figures in  $\mu$ M; e.g., in Fig. 2, "C 0.1" indicates 0.1  $\mu$ M of invention compound 21. Similar analyses (data not shown) were also conducted for the majority of the compounds in Table 1.

For the majority of the dimeric binding compounds, regions of binding (footprints) generally overlapped those shown by the control compounds (distamycin or netropsin) but covered larger regions of DNA. Clear footprints of 8-11 bases were typical, though apparent footprints of 20-30 bases were observed, e.g. for compounds 21 and 25. Target sequences were generally A/T rich, as expected, though footprinting was often observed for regions with 50% or more G/C content. For some compounds, as noted below, footprinting was seen at regions where none was apparent for the control compounds. Less frequently (e.g. for compound 25), footprinting was not observed at regions where distamycin did give a footprint.

Binding affinities of the dimers were evaluated based on comparison of the concentrations at which the dimers produced clear footprinting, in comparison to the control compound. On this basis, binding affinity could be ordered roughly as follows, using the compound

designations above: 21, 25 > 46, 45 > 26, 52, 50 > 49, 32, 51 > 48.

Compounds 21 (2,5-indolyl linker) and 25 (9,10-dihydro-2,7-phenanthrenyl linker) clearly showed higher binding affinities than the control compounds, giving clear footprints at much lower concentrations. Selected examples are given in Table 2 for Compound 21. (In the fifth entry, the two sequences listed are adjacent.)

Table 2

Footprinted Sequence	SEQ ID NO:	Cmpd 21 Conc. ( $\mu$ M)	Distamycin Conc. ( $\mu$ M)
AGGAAAA (IL-2)	4	0.1	3 - 12
CAAAG (IL-2)	5	0.01 - 0.1	3 - 12
AGTTACT (IL-2)	6	0.1	3 - 12
AGGTATTC (IL-1 $\beta$ )	7	1	25 - 50
TCTGCTTTTGAA; AGCTATAAAA (IL-1 $\beta$ )	8; 9	6; 0.1 - 1	> 50; 1 - 12

For compound 25, binding was generally seen at concentrations of 0.1 to 1  $\mu$ M for sequences at which distamycin footprinting was seen at 25-50  $\mu$ M, as shown in Table 3. Footprinting was also observed at several sequences at which no footprinting was seen with distamycin (e.g. CACTTG, CAGGC in IL-1 $\alpha$ ; TTAATT, AAGCTT, GGCAGATACCA in IL-1 $\beta$ ) (SEQ ID NOs: 10-15, respectively).

Table 3

Footprinted Sequence (IL-1 $\alpha$ )	SEQ ID NO:	Cmpd 25 Conc. ( $\mu$ M)	Distamycin Conc. ( $\mu$ M)
AATACAAAA	15	0.1 - 1	25 - 50
CCCTGTAA	16	0.1 - 1	25 - 50
AGACAATTACA	17	0.1 - 1	12 - 25
GCGAAGAAG	18	1 - 6	100

Compound 46 (phenanthrene linker) and prior art compound 45 (*trans*-vinyl linker) were run side by side with netropsin and showed footprinting in similar regions but at lower concentrations than netropsin; e.g. typically 0.1 to 1  $\mu$ M (45) vs 6.25  $\mu$ M (netropsin) ; 1  $\mu$ M (46) vs 25  $\mu$ M (netropsin). Compound 46 also showed footprinting, at fairly high concentrations (about 50  $\mu$ M), at sequences where distamycin did not appear to bind (e.g. ATCACTCTCTTAAT in IL-2) (SEQ ID NO: 19).

Compounds 32 and 49 (3,7-benzothiophenyl and 3,7-benzofuranyl linkers, respectively) gave footprinting in selected regions of IL-2 (e.g. TAATATTTTCCA at 0.1  $\mu$ M, compound

49; TTTAATCACTACT at 0.1  $\mu$ M, compound 32) (SEQ ID NOs: 20-21, respectively). The overall footprinting intensity was qualitatively similar to that of the control compounds. An initial run of prior art compound 48 (cyclopropyl linker) gave no discernable footprinting; however, this result was not fully conclusive.

5 Compounds 51 and 52 (9,10-dihydro-2,7-phenanthrenyl linker with morpholino and piperazinyl end groups, respectively) and prior art compound 50 (m-phenyl linker) showed footprints at regions of IL-2 generally similar to those observed with distamycin. The binding affinity of 51 appeared to be comparable to that of distamycin. Compound 52 gave footprints at concentrations of 1-12 or 12-50  $\mu$ M, depending on sequence; compound 50, run side by side  
10 with 52, bound at similar regions but footprinting was less pronounced.

As the data described above shows, the netropsin dimers of the invention bind to DNA with an affinity that is at least comparable, and in some cases greatly exceeds, that of the monomeric compounds netropsin and distamycin. Certain invention compounds exhibited significantly greater binding affinities than compounds of the prior art, and in some cases exhibited binding to  
15 regions not observed with the control compounds and prior art compounds.

## EXAMPLES

The following examples illustrate but are not intended to limit the invention.

20 Example 1. Preparation of Compound 21 (N,N'-di{1-methyl-2-[1-methyl-2-(1-methyl-2-carboxamide(3-amidino)-4-pyrrole)-carboxyamido-4-pyrrole]carboxamido-4-pyrrolyl}-indolyl-2,5-dicarboxamide)

A distamycin derivative having a 4-nitropyrrole at one terminus and a 2-(carboxamidopropionitrile)pyrrole on the other terminus (1-methyl-4-(1-methyl-4-aminopyrrole-2-carboxamido)-pyrrole-2-carboxamidopropionitrile) was prepared according to the procedure of  
25 Lown *et al.*, 1985. The nitrodimer compound in ethanol was hydrogenated over Pd/C-10% (1 equivalent w/w) at 60°C for 5h. The catalyst was removed by filtration and the solvent removed under reduced pressure to yield the amine, which was used immediately without further purification. To a solution of three equivalents of the amine in DMF under nitrogen were added one equivalent of indole-2,5-dicarboxylic acid and catalytic amounts of dimethylaminopyridine  
30 and HOBT, and the reaction was cooled on ice. A solution of 4.5 equivalents of DCC in DMF was added slowly, and the reaction was allowed to warm to room temperature, and then stirred for 1-5 days. Water was added and the precipitated DCU removed by filtration. The filtrate was concentrated under reduced pressure, and the product isolated by high performance liquid chromatography.

35 <sup>1</sup>H-NMR (CD<sub>3</sub>OD): 8.29 (d, 1H, indole H-7, J<sub>H7-H5</sub> = 1.5 Hz); 7.82 (m, 1H, indole H-5, J<sub>H5</sub>.

$H_4 = 9.0$  Hz); 7.57 (m, 1H, indole H-4); 7.33 (s, 1H, indole H-2); 7.29, 7.15, 7.05, 6.91 (m,d,d,m; 2H, 2H, 2H, 2H; pyrrole H- $\alpha$ , H- $\beta$ ); 3.94 (s, 3H, pyrrole N-Me); 3.89 (s, 3H, pyrrole N-Me); 3.65 (s, 2H,  $NHCH_2CH_2C(=NH)NH_2$ ); 2.72 (s, 2H,  $NHCH_2CH_2C(=NH)NH_2$ ).

5

Example 2. Preparation of Compound 46 (N,N'-di{1-methyl-2-[1-methyl-2-(1-methyl-2-carboxamide(3-dimethylaminopropyl)-4-pyrrole)-carboxyamido-4-pyrrole]carboxamido-4-pyrrolyl}-phenanthrenyl-2,7-dicarboxamide)

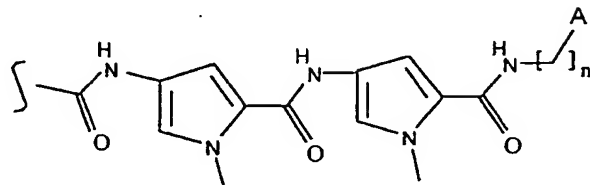
Nitrodistamycin, prepared according to reported procedures (e.g. Nishiwaki *et al.*, 1988), was reduced to the amino derivative by catalytic hydrogenation. The nitro compound was dissolved in 1:1 DMF/MeOH and treated with 10% Pd/C (100 mg / 200 mg nitrodistamycin) for 2 hrs at room temperature, 40 psi. The amino compound was recovered by filtration and evaporation of the solvent. A mixture of phenanthrene-2,7-dicarboxylic acid, three equivalents of HBTU, and six equivalents of NMM in dry DMF were stirred under nitrogen at room temperature for 30 minutes. Three equivalents of the amine in DMF were added and the reaction stirred for 18h. The reaction mixture was concentrated and the product isolated by standard chromatographic methods.

10

15

It is claimed:

1. A DNA binding compound having the structure B-L-B', where B and B' independently have the structure



5

in which

A is amidinyl ( $-C(NH_2)=NH$ ) or  $-CH_2NRR'$ , where R and R' are independently hydrogen or lower alkyl, or together form a 5- to 7-member heterocyclic ring whose ring atoms are selected from the group consisting of carbon, nitrogen, oxygen and sulfur, and include at least 3 carbon

10

atoms;

n is 1-6;

L is a fused polycyclic ring system containing two or more fused 5- to 7-membered rings, and containing at least one aromatic ring; and

each of groups B and B' is linked to a different ring of said ring system, such that the shortest path connecting groups B and B' comprises at least three contiguous bonds within the ring system when the groups are on adjacent fused rings, and comprises at least five contiguous bonds within the ring system when the groups are on non-adjacent rings.

15

2. A compound as in claim 1, wherein said ring system is bicyclic or tricyclic.

20

3. A compound as in claim 1, wherein B and B' are identical.

4. A compound as in claim 3, wherein  $n = 2$ .

25

5. A compound as in claim 4, wherein A is amidinyl or (dimethylamino)methyl.

6. A compound as in claim 5, wherein A is amidinyl.

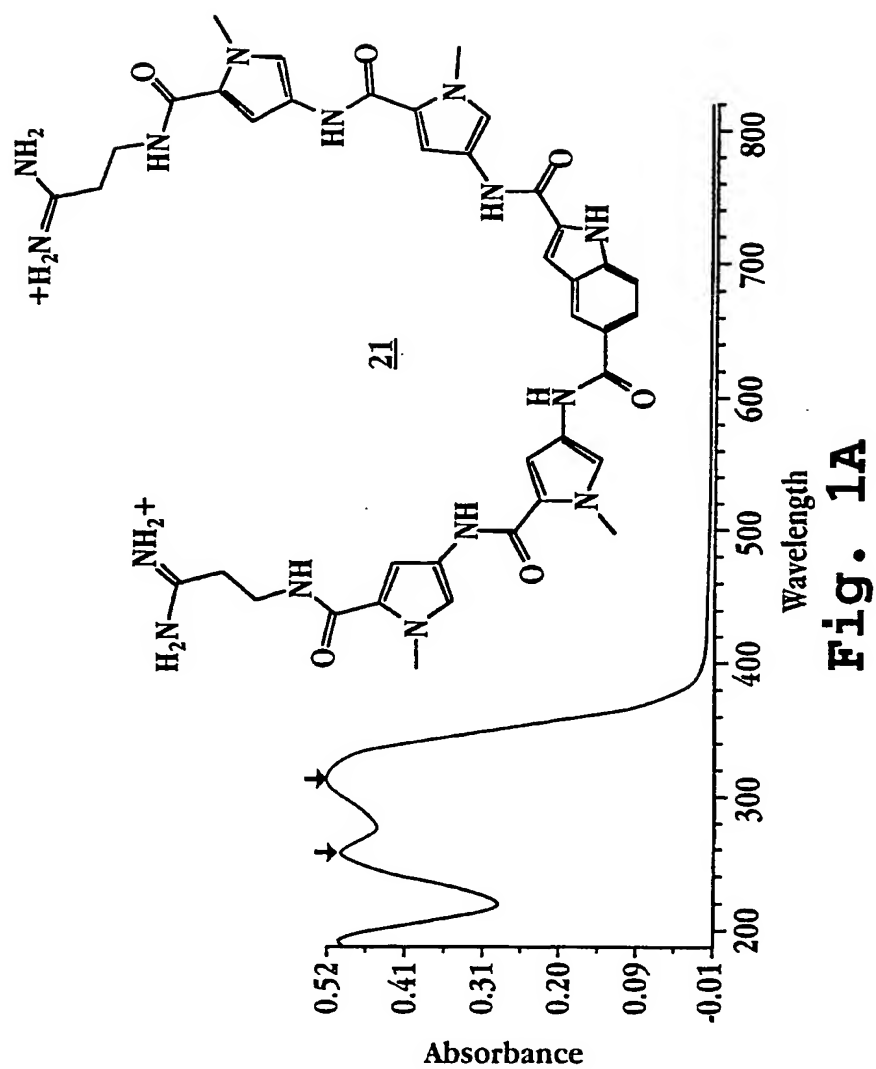
7. A compound as in claim 1, wherein L is 2,5-indolyl.

30

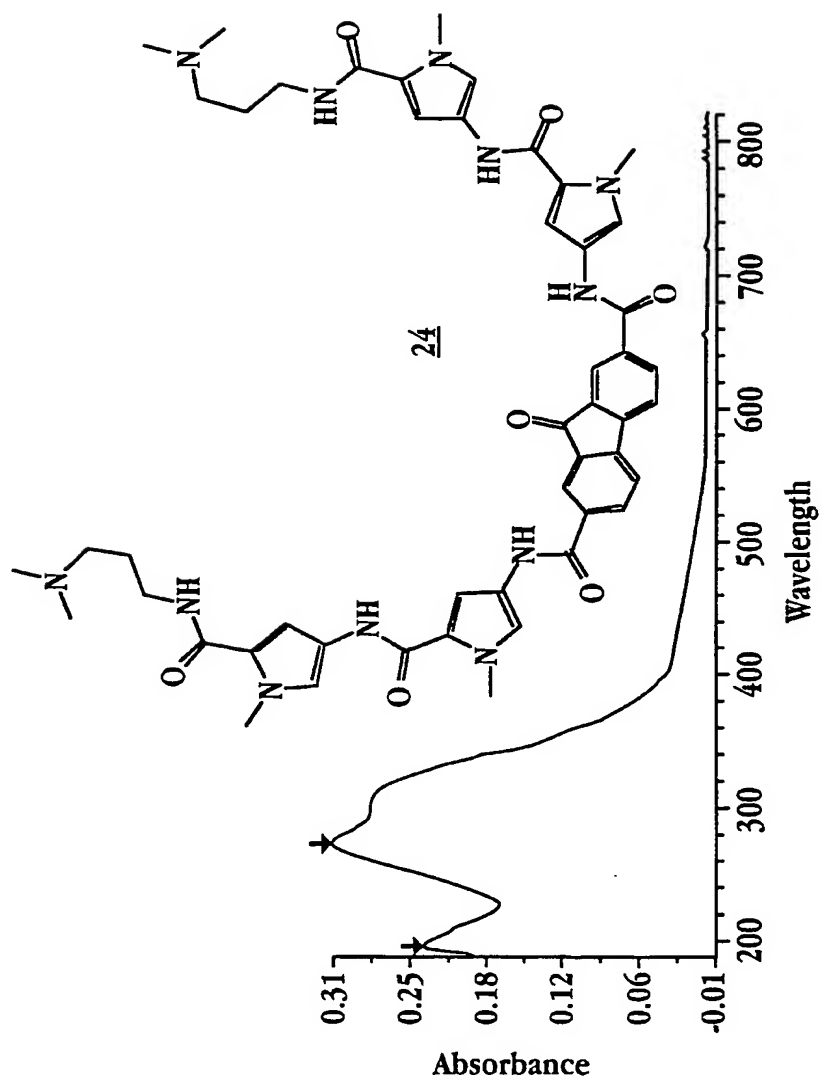
8. A compound as in claim 5, wherein L is 2,5-indolyl.

9. A compound as in claim 6, wherein L is 2,5-indolyl.
10. A compound as in claim 1, wherein L is 9,10-dihydro-2,7-phenanthrenyl.
- 5 11. A compound as in claim 5, wherein L is 9,10-dihydro-2,7-phenanthrenyl.
12. A compound as in claim 6, wherein L is 9,10-dihydro-2,7-phenanthrenyl.
13. A compound as in claim 5, wherein L is 2,6-indolyl.
- 10 14. A compound as in claim 5, wherein L is 2,7-phenanthrenyl.
15. A compound as in claim 5, wherein L is 3,7-dibenzofuranyl.
- 15 16. A compound as in claim 5, wherein L is 3,7-dibenzothiophenyl.
17. A compound as in claim 5, wherein L is 3,7-fluorenonyl.
18. A compound as in claim 5, wherein L is 6-hydroxy-dibenzo[d,f][1,3,2]dioxaphosphepin-6-  
20 oxide.

1/20

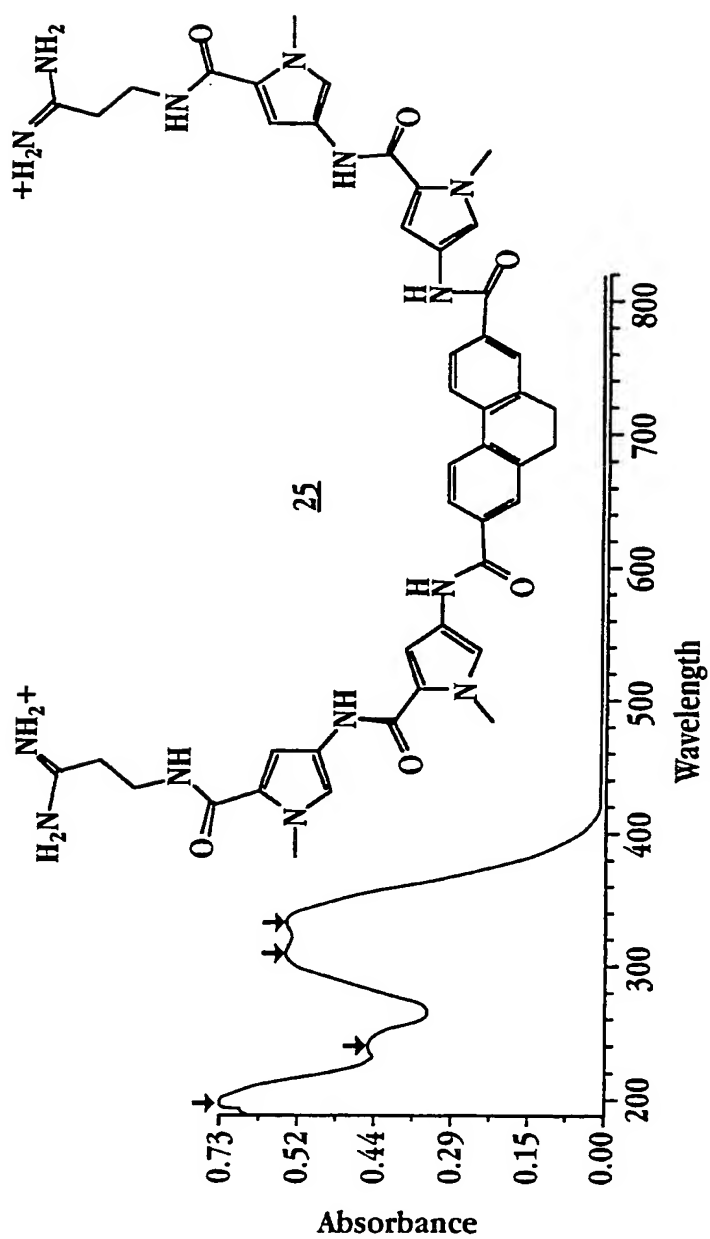


2 / 20

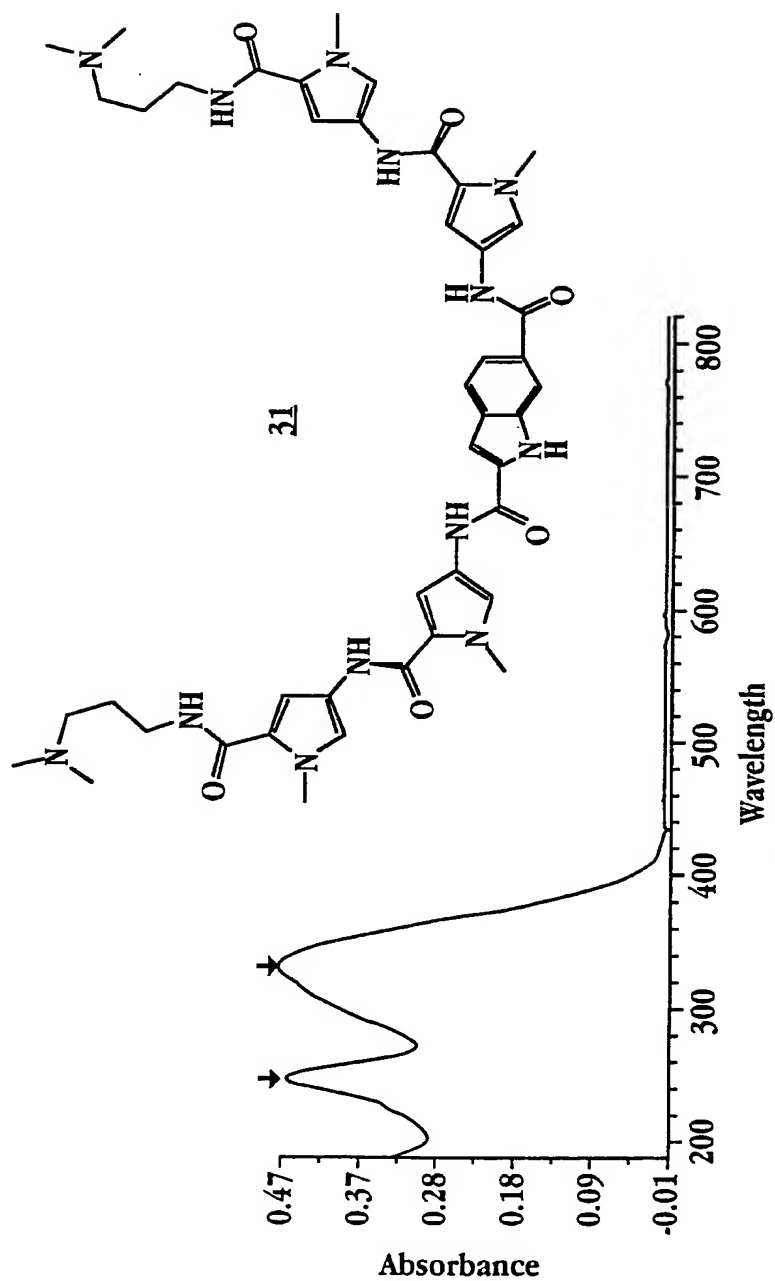
**Fig. 1B**



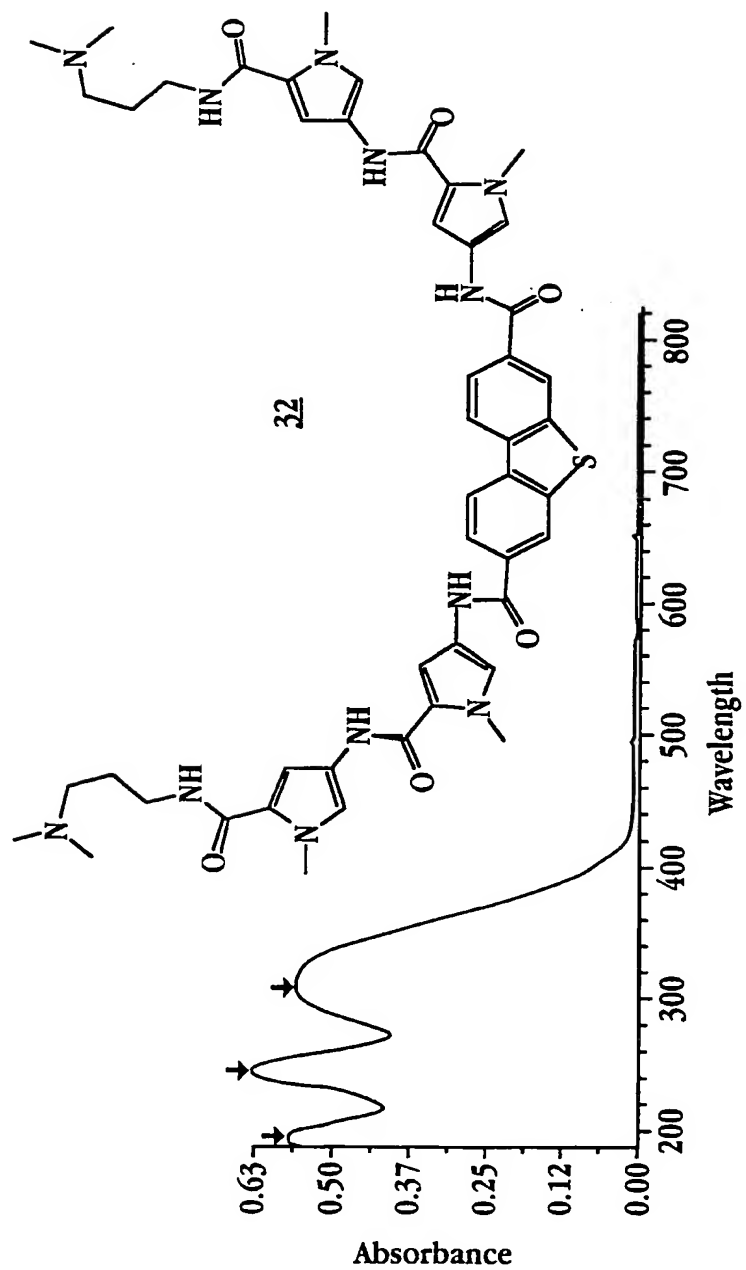
3/20

**Fig. 1C**

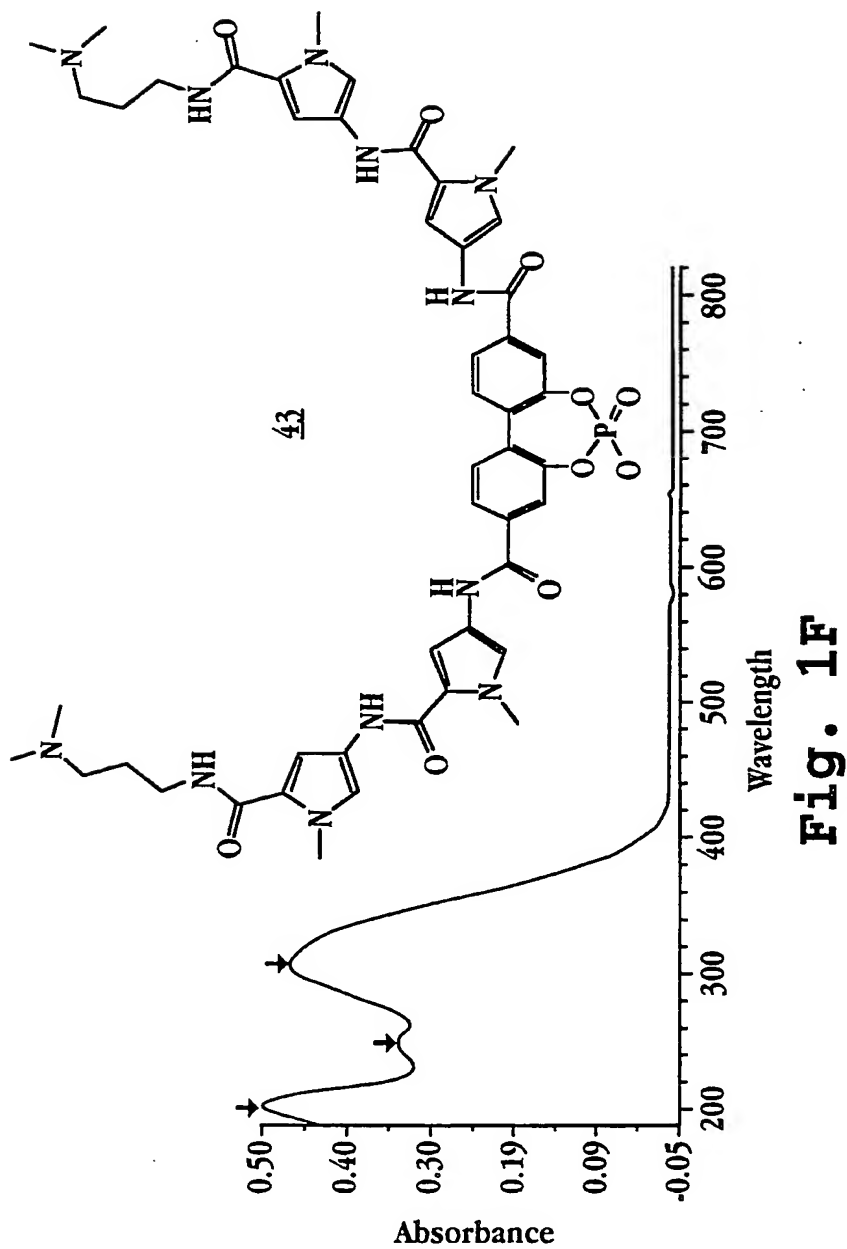
4/20

**Fig. 1D**

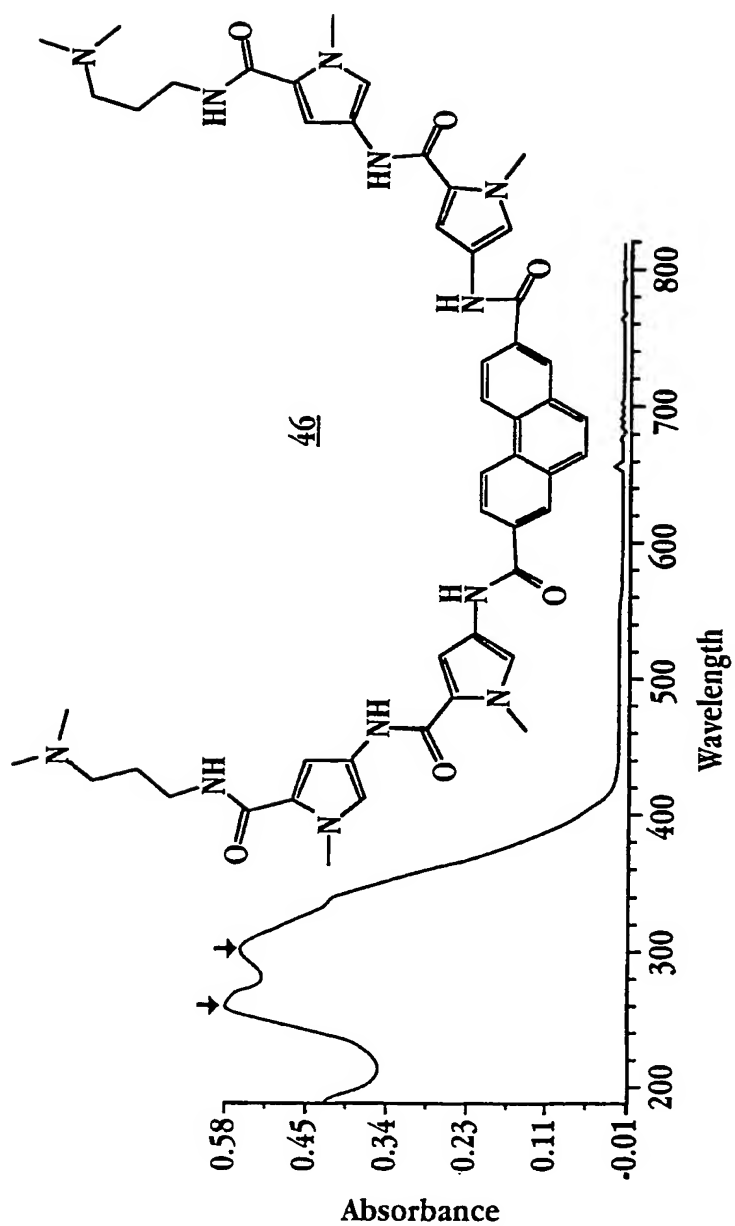
5/20

**Fig. 1E**

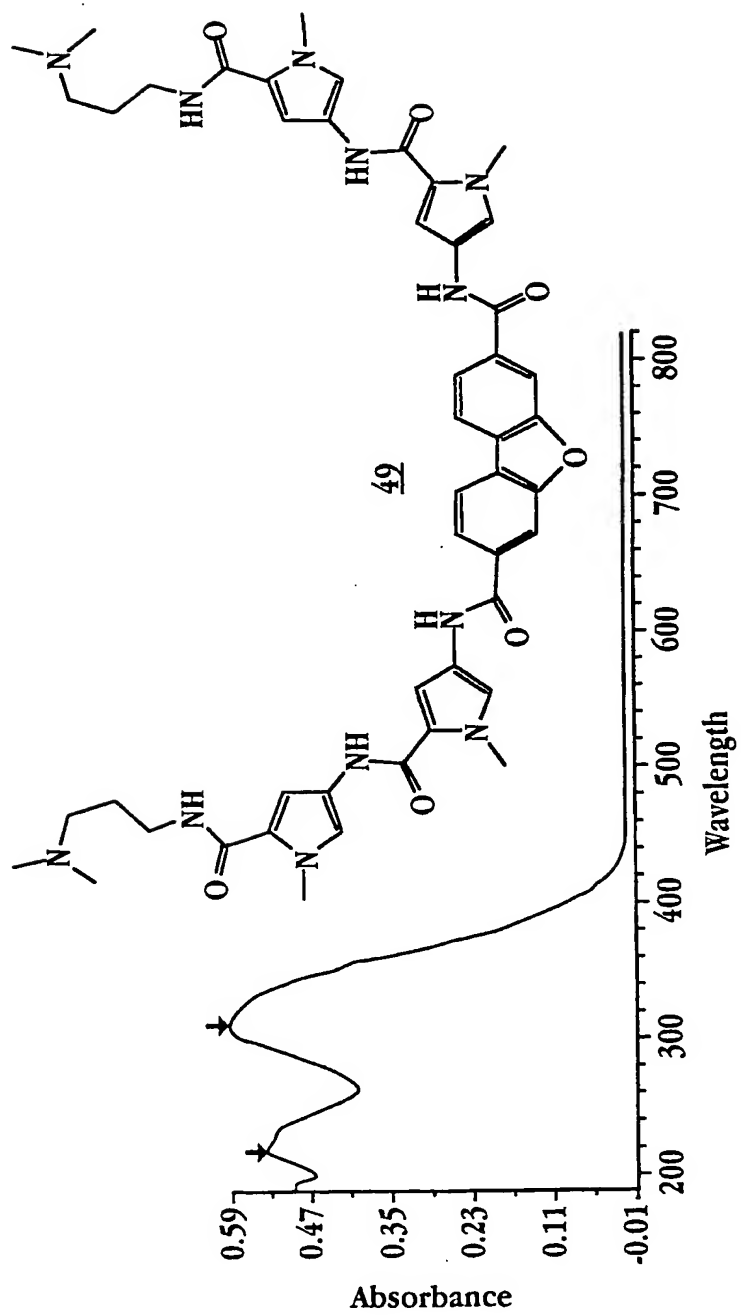
6/20

**Fig. 1F**

7/20

**Fig. 1G**

8/20

**Fig. 1H**

9/20

	T	T	G	A	A	T	G	A	A	C	A	T	A	G	A	A	A	A	T	A	C	A	A	A	C	T	G	A
D 50																												
D 12.5																												
D 3.1																												
C 25																												
C 6.2																												
C 1.0																												
C 0.1																												
C 0.01																												

	A	T	T	C	T	T	C	C	C	T	G	T	A	A	A	T	T	C	C	C	C	G	T	T	T	T	G	A	C	G
D 50																														
D 12.5																														
D 3.1																														
C 25																														
C 6.2																														
C 1.0																														
C 0.1																														
C 0.01																														

Legend	
	Footprint
	Partial footprint
	No band observed
	Enhanced cleavage

Fig. 2A

10/20

	A	C	G	C	A	C	T	T	G	T	A	G	C	C	A	C	G	C	C	T	A	C
D 50																						
D 12.5																						
D 3.1																						
C 25	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
C 6.2	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
C 1.0																						o
C 0.1																						
C 0.01																						

	T	T	A	A	G	A	C	A	A	T	T	A	C	A	A	A	A	G	C	G	A	A	G	A	A	G
D 50	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
D 12.5	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
D 3.1																										
C 25	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
C 6.2	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
C 1.0	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
C 0.1	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
C 0.01																										

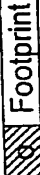
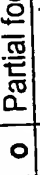


Legend
 Footprint
 Partial footprint
 No band observed
 Enhanced cleavage

Fig. 2B



11/20

	C	C	T	A	A	G	A	A	G	C	T	T	C	C	A	C	C	A	A	T	A	C	T	C	T	T	T	C	C	C
D 50																														
D 25																														
D 12.5																														
D 1.0																														
C 12.5																														
C 6.2																														
C 1.0																														
C 0.1																														
C 0.01																														

	C	T	T	C	C	T	T	A	A	C	T	T	A	A	C	T	T	G	A	T	T	G	T	G	A	A	T	C	A	G	T
D 50																															
D 25																															
D 12.5																															
D 1.0																															
C 12.5																															
C 6.2																															
C 1.0																															
C 0.1																															
C 0.01																															

Legend	
	Footprint
	Partial footprint
	No band observed
	Enhanced cleavage

Fig. 3A

12/20

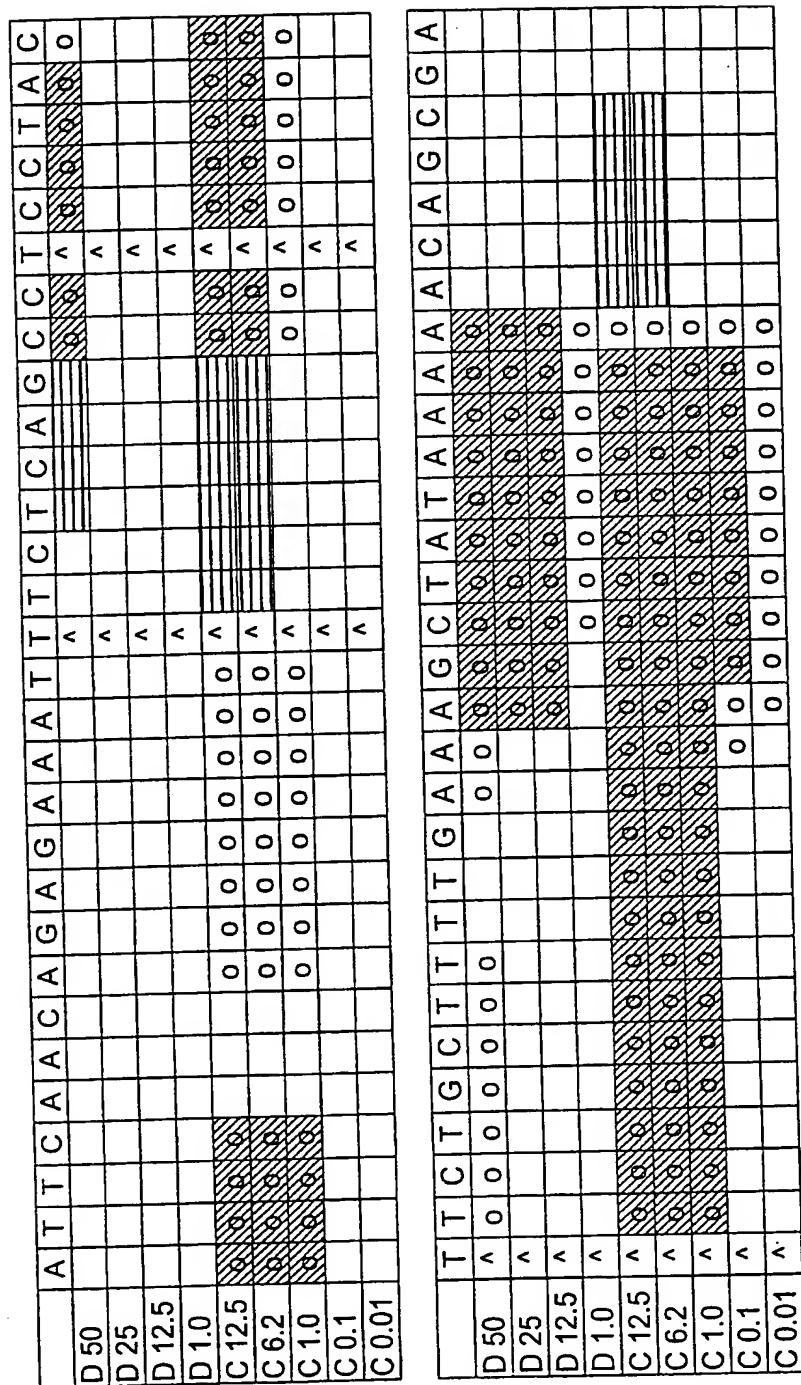


Fig. 3B

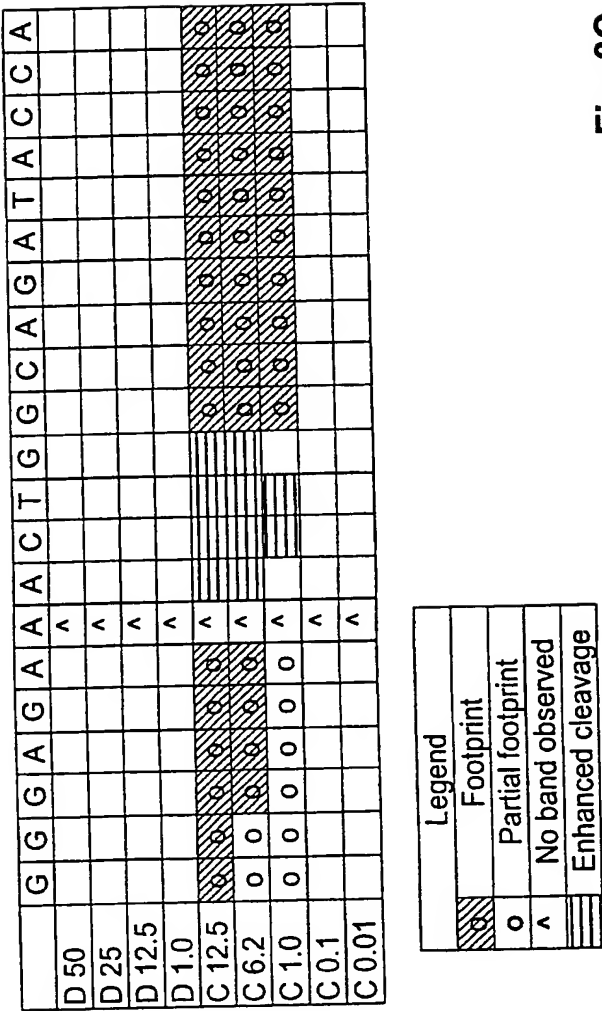


Fig. 3C

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	G	T	A	G	T	T	T	A	T	G	A	C	A	A	A	G	A	A	A	T	T	T	C	T	G	A
D50	^	o	o	^	^	^	o	o	^	^	^	o	o	o	^	^	^	^	^	^	o	^	^	^	o	
D12.5	^	o	o	^	^	^	o	o	^	^	^	o	o	o	^	^	^	^	^	^	o	^	^	^	o	
D3.1	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	
D1.0	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	
C12.5	^	o	o	^	^	^	o	o	^	^	^	o	o	o	o	o	o	o	o	^	^	o	o	o	o	
C6.2	^	o	o	^	^	^	o	o	^	^	^	o	o	o	o	o	o	o	o	^	^	o	o	o	o	
C1.0	^	o	o	^	^	^	o	o	^	^	^	o	o	o	o	o	o	o	o	^	^	o	o	o	o	
C0.1	^	^	^	^	^	^	o	o	^	^	^	o	o	o	o	o	o	o	o	^	^	o	o	o	o	
C0.01	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	

	G	T	A	C	T	T	T	T	G	T	A	T	C	C	C	A	C	C	C	C	C	T	T	A	A	G	A
D50	o	o	o	o	o	o	o	o	^	o	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	
D12.5	o	o	o	o	o	o	o	o	^	o	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	
D3.1	o	o	o	o	o	o	o	o	^	o	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	
D1.0	o	o	o	o	o	o	o	o	^	o	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	
C12.5	o	o	o	o	o	o	o	o	^	o	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	
C6.1	o	o	o	o	o	o	o	o	^	o	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	
C1.0	o	o	o	o	o	o	o	o	^	o	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	
C0.1	o	o	o	o	o	o	o	o	^	o	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	
C0.01	o	o	o	o	o	o	o	o	^	o	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	





Legend	
	Footprint
	Partial footprint
	No band observed
	Enhanced cleavage

Fig. 4A

	A	A	G	G	A	G	A	G	A	A	A	A	A	A	C	T	G	T	T	C	A	T	A	C	A	G	A	G	G	C
D50					o	o																				o	o	o	o	o
D12.5					o	o																			o	o	o	o	o	o
D3.1					o	o																			o	o	o	o	o	o
D1.0					o	o																			o	o	o	o	o	o
C12.5					o	o																			o	o	o	o	o	o
C6.2					o	o																			o	o	o	o	o	o
C1.0					o	o																			o	o	o	o	o	o
C0.1					o	o																			o	o	o	o	o	o
C0.01					o	o																			o	o	o	o	o	o

	G	T	T	A	A	T	G	C	A	T	G	A	T
D50	o	o	o	o	o	o	o	o	o	o	o	o	o
D12.5	o	o	o	o	o	o	o	o	o	o	o	o	o
D3.1													
D1.0													
C12.5	o	o	o	o	o	o	o	o	o	o	o	o	o
C6.2	o	o	o	o	o	o	o	o	o	o	o	o	o
C1.0	o	o	o	o	o	o	o	o	o	o	o	o	o
C0.1	o	o	o	o	o	o	o	o	o	o	o	o	o
C0.01													





Legend	
	Footprint
	Partial footprint
	No band observed
	Enhanced cleavage

Fig. 4B

16/20

	T	T	G	A	A	T	G	A	T	G	A	A	C	A	T	A	C	A	A	C	T	G	A
D 100	o	o	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^
D 50			^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^
D 25			^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^
C 12.5			^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^
C 6.2	o	o	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^
C 1.0	o	o	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^
C 0.1	o	o	^	o	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^
C 0.01			^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^
C 0.001			^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^	^

	A	T	T	C	T	T	C	C	C	T	G	T	A	A	T	T	C	C	C	G	T	T	T	G	A	C	G
D 100	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
D 50	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
D 25	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
C 12.5																											
C 6.2	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	
C 1.0	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	
C 0.1	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	
C 0.01	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	
C 0.001																											

Legend	
o	Footprint
o	Partial footprint
^	No band observed
o	Enhanced cleavage

Fig. 5A

SUBSTITUTE SHEET (RULE 25)

	A	C	G	C	A	C	T	G	T	A	G	C	C	A	C	G	C	C	T	A	C
D 100																					
D 50																					
D 25																					
C 12.5																					
C 6.2																					
C 1.0																					
C 0.1																					
C 0.01																					
C 0.001																					

	T	T	A	G	A	C	A	A	T	T	A	C	A	A	A	G	G	C	G	A	A	G	A	A	G	A	C	T	G	A	C	T	C	A	G	G	C
D 100																																					
D 50																																					
D 25																																					
C 12.5																																					
C 6.2																																					
C 1.0																																					
C 0.1																																					
C 0.01																																					
C 0.001																																					

SUBSTITUTE SHEET (RULE 26)





Legend	
	Footprint
	Partial footprint
	No band observed
	Enhanced cleavage

Fig. 5B

	C	T	A	G	A	G	C	T	T	C	A	C	C	A	T	A	C	T	C	T	T	T	C	C	C
D 50	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
D 25	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
D 12.5	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
D 1.0	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
C 12.5	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
C 1.0	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
C 0.1	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
C 0.01	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o

	C	T	T	T	C	G	T	T	T	A	A	C	T	T	G	A	T	T	G	T	G	A	A	T	C	A	G	G	T
D 50	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
D 25	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
D 12.5	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
D 1.0	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
C 12.5	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
C 1.0	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
C 0.1	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
C 0.01	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o

SUBSTITUTE SHEET (RULE 26)

Legend
Footprint
Partial footprint
No band observed
Enhanced cleavage

Fig. 6A



	A	T	T	C	A	A	C	A	G	A	G	A	A	T	T	T	C	T	C	A	G	C	C	T	C	C	T	A	C
D 50	o	o	o	o	o	o	o														^	o	o	o	o	o	o	o	o
D 25																					^								
D 12.5																					^								
D 1.0																					^								
C 12.5	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	^	o	o	o	o	o	o	o	o
C 1.0	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	^								
C 0.1							o	o	o	o	o	o	o	o	o	o	o	o	o	o	^								
C 0.01							o	o	o	o	o	o	o	o	o	o	o	o	o	o	^								

	T	T	C	T	G	C	T	T	T	T	G	A	A	A	G	C	T	A	T	A	A	A	A	C	A	G	C	G	A
D 50	^	o	o	o	o	o	o	o			o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
D 25	^											o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	
D 12.5	^											o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	
D 1.0	^											o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	
C 12.5	^	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
C 1.0	^	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
C 0.1	^											o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
C 0.01	^											o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o

Legend	
	Footprint
	Partial footprint
	No band observed
	Enhanced cleavage

Fig. 6B

	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171
	G	G	G	A	G	A	A	A	C	T	G	G	C	A	G	A	T	A	C	C	A
D 50							^														
D 25							^														
D 12.5							^														
D 1.0							^														
C 12.5	o	o	o	o	o	o	^														
C 1.0	o	o	o	o	o	o	^									o	o	o	o	o	o
C 0.1																					
C 0.01																					





Legend	
	Footprint
	Partial footprint
	No band observed
	Enhanced cleavage

Fig. 6C

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 00/25325

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 C07D207/34 C07D403/14 C07D409/14 C07D405/14 C12N15/09

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 C07D C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)  
EPO-Internal, CHEM ABS Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	KHALAF, A. I. ET AL: "The synthesis of some head to head linked DNA minor groove binders" TETRAHEDRON., vol. 56, no. 29, 14 July 2000 (2000-07-14), pages 5225-5239, XP002158867 ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM., NL ISSN: 0040-4020 the whole document	1-18
X	US 4 942 227 A (DERVAN PETER B ET AL) 17 July 1990 (1990-07-17) the whole document	1

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

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\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search

30 January 2001

Date of mailing of the international search report

21/02/2001

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Authorized officer

Bosma, P

# INTERNATIONAL SEARCH REPORT

Internat. Application No

PCT/US 00/25325

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; YAVORSKAYA, N. P. ET AL: "Search for antitumor compounds among actinocin derivatives" retrieved from STN Database accession no. 127:229198 XP002158869 abstract; CAS RN 195307-24-5 & KHIM.-FARM. ZH. (1996), 30(12), 22-26 ,	1-5
A	----- SINGH, MALVINDER P. ET AL: "Isohelicity and Strand Selectivity in the Minor Groove Binding of Chiral (1R,2R)- and (1S,2S)-Bis(netropsin)-1,2-cyclopropanedic arboxamide Ligands to Duplex DNA" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY., vol. 116, no. 16, 1994, pages 7006-7020, XP002158868 AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC., US ISSN: 0002-7863 figure 8	1
A	----- US 5 273 991 A (LEE MOSES N F) 28 December 1993 (1993-12-28) claims 1,20,21; examples	1
A	----- FR 2 666 088 A (CENTRE NAT RECH SCIENT ;ROUSSY INST GUSTAVE (FR)) 28 February 1992 (1992-02-28) claims 1,5,10,11; examples -----	1

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Information on patent family members

International Application No

PCT/US 00/25325

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FR 2666088 A	28-02-1992	NONE	